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Synthesis of Oxa Analogs of Porphobilinogen (PBG) as Probes for Mechanistic Studies of PBG Deaminase§

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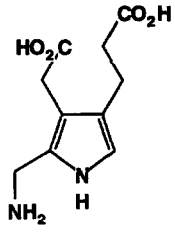
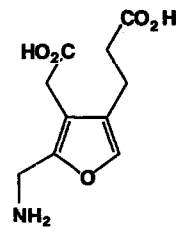
Abstract: Three oxa analogs of porphobilinogen (PBG) **2**, **27**, and **29** were synthesized from 3-hydroxymethylfuran. The target molecules were prepared by introducing first the β-acetate sidechain, then constructing propionate side chains on the furan nucleus into which the aminomethyl group was introduced at position 2 via the corresponding aldehyde.

Porphobilinogen (PBG) **1** (Scheme I) is a key compound in the biosynthesis of porphyrin rings in nature. Numerous PBG analogs have been prepared and tested as substrates for PBG deaminase, the polymerizing enzyme of tetrapyrrole biosynthesis and most of these were constructed from substituted pyrrole rings, with the exception of two furans **3** and **5** and two thiophenes **4** and **6** (Scheme II). Compounds **3** and **4** were reported to be weak non-competitive inhibitors of deaminase, with K_i values ranging from 63-170 nM.[1] Since it was of interest to study whether an oxa analog of PBG would be recognized by the enzyme PBG deaminase, we undertook the synthesis of the PBG oxa analog **2** to probe the mechanism of the enzyme with the following goals: (1) to determine whether **2** is a substrate or inhibitor, (2) is oxa PBG recognized at all by the enzyme, (3) if **2** is a substrate, the enzymatic reaction of PBG deaminase/uro'gen III synthetase could be used to prepare the oxa analogs of type III porphyrins biochemically. Oxa porphyrins have been synthesized recently by Vogel[2] and were found to be stable.

§ Dedicated, with respect and best wishes, to Sir Derek Barton on the occasion of his 75th birthday

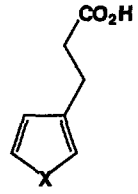


PBG 1

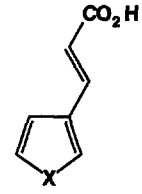


OXA PBG 2

Scheme I



3 X = O
4 X = S

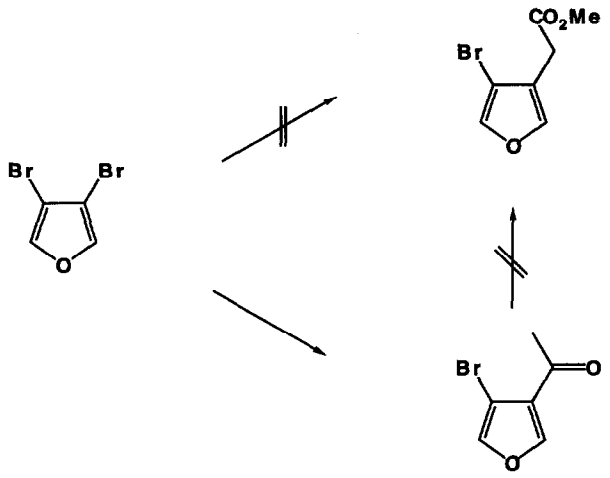


5 X = O
6 X = O

Scheme II

Synthetic furans have been employed either as substructures in the total synthesis of natural products or as intermediates for conversion into polyfunctionalized chain compounds. Furans can be alkylated at positions 2 and 5 via the corresponding lithiated intermediates, prepared directly using organometallic reagents such as n-butyl lithium. Preparation of furans substituted in position 3 and 4 have required new methods, most of which have been developed in the last ten years.[3-8] Recently Danheiser[9] has demonstrated that allenylsilanes can be transformed into 3-, and 4-substituted furans in high yield, and Marshall[10] has described a new route to 2,3,5-trisubstituted furans from allenyl ketones. It is well known that the furan ring is reduced, oxidized and cleaved under relatively mild conditions, and hence we have aimed at the necessary introduction of substituents at position 3 and 4 with the proper oxidation state installed.

Our first approach to the synthesis of the trisubstituted furan target molecule **2** started with the known 3,4 dibromofuran[11] with the idea of introducing the carboxymethyl group at the first stage as shown in Scheme III. It is known that halogen-lithium exchange can be carried out on 3-halofurans[12] by n-butyl lithium at low temperature. We followed this approach and alkylated the lithiated furan with methyl bromoacetate. Unfortunately none of the expected product could be isolated from the reaction mixture. Oxidative

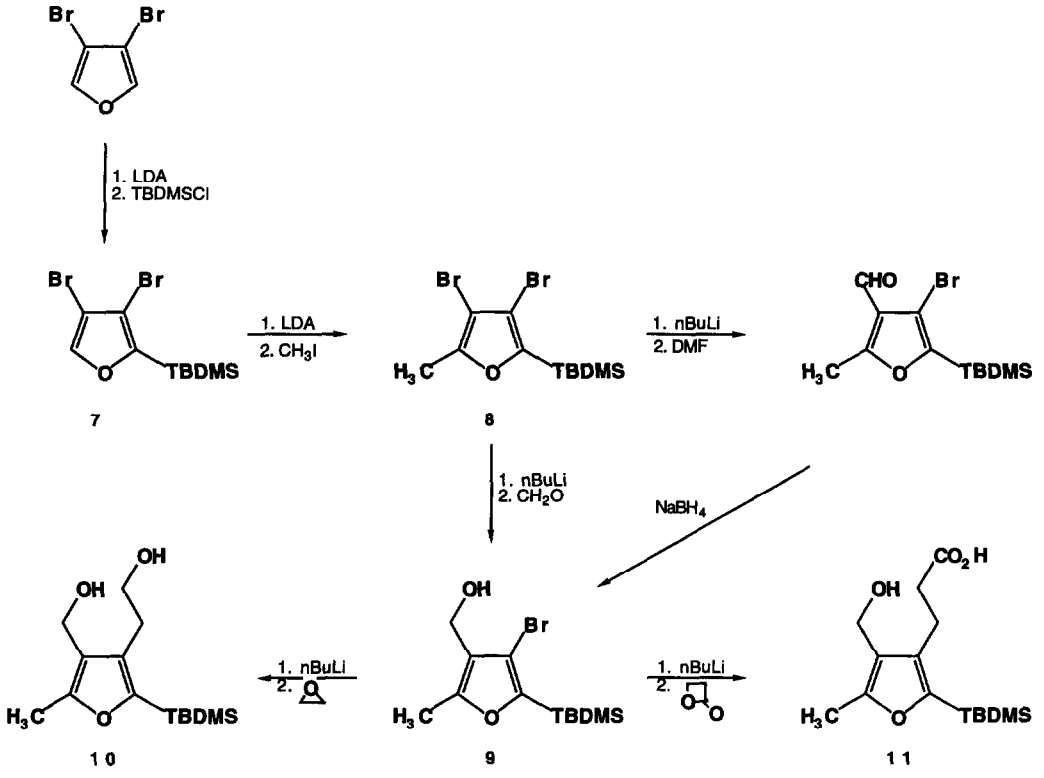


Scheme III

rearrangement is known to be the key step in the conversion of an acylpyrrole to the corresponding pyrrole acetate using either thallium (III) nitrate[13] or silver nitrate;[14] similar results have been obtained with thiophens.[15] Reaction of 3,4-dibromofuran with n-butyl lithium at -78 °C, followed by exchange of lithium by magnesium[16] and acylation with acetic anhydride produced 3-bromo-4-acetylfuran in 70% yield. All attempts to convert this acyl furan to the corresponding acid were unsuccessful. While this work was being carried out we became aware of a report that treatment of 2-acylfuran with thallium nitrate produces the corresponding acid in poor yield (23%)[17] and therefore this route was also abandoned.

We next decided to change strategies and synthesize the target molecule **2** having substituents at positions 2 and 5, one of which is a methylgroup that can be converted to the aminomethyl side-chain and the other a bulky blocking group removable at the appropriate state. The bulk of the silyl protecting group could serve for directing selective halogen lithium exchange in the 3,4 dibromo intermediate. Treatment of 3,4-dibromofuran using lithium diisopropylamide (LDA), and silylation of the lithiated intermediate with t-butyldimethylsilyl chloride give the 2-(t-butyldimethylsilyl)-3,4-dibromofuran **7** (Scheme IV) in over 90% isolated yield. Further lithiation of **7**, again using LDA and alkylation with methyl iodide gave **8** in 90% yield. We then planned to introduce the acetate chain in two steps: hydroxymethylation followed by nucleophilic substitution of the hydroxyl by cyanide. It was found that when **8** was lithiated with n-butyl lithium and treated with either freshly prepared formaldehyde or with dimethylformamide, followed by reduction with sodium borohydride the expected 2-(t-butyldimethylsilyl)-4-bromo-3-hydroxymethyl-5-methyl furan **9** was

formed in only 20-30% yield. All attempts to improve the yield by varying the reaction conditions failed. The next step turned out to be even worse, for when we tried to introduce the propionate side chain by reaction on lithiated **9** either with ethylene oxide or with β-propiolactone,[18] in both cases only a trace of **10** or **11** respectively could be isolated. Protection of the hydroxy group by MEM did not improve the yield, and therefore this approach was abandoned.

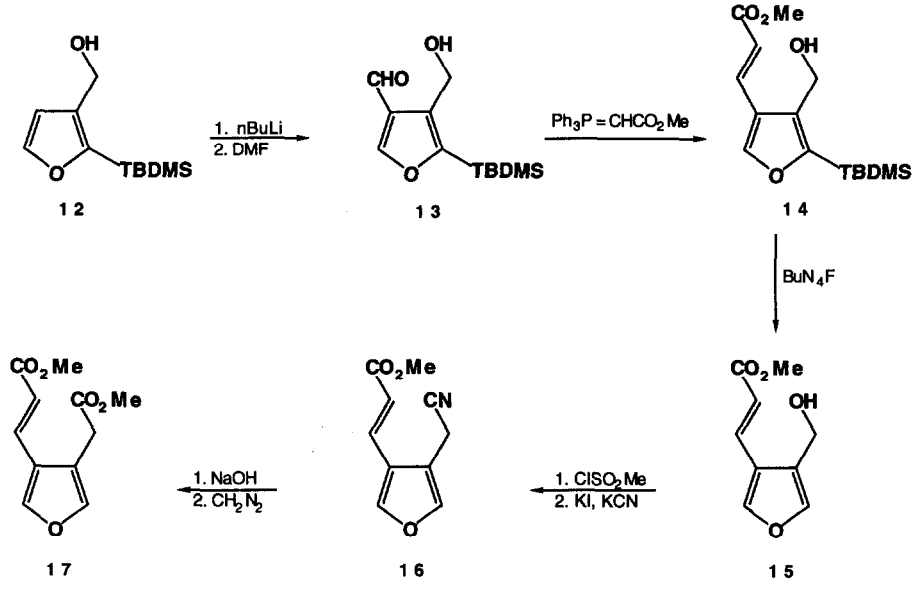


Scheme IV

The next attempt was based on introduction of side chains at positions 3 and 4 carrying carboxylate functions as described in scheme V, followed by introduction of the aminomethyl function at position 2. As starting material we chose the commercially available 3-hydroxymethylfuran which was converted[5] in 92% yield to 2-(t-butyldimethylsilyl)-3-hydroxymethyl furan **12** via a 1,4 O→C rearrangement. Since it had been shown by Keay[5] that treatment of **12** with n-butyl lithium leads to metalation at position 4 we were able to formylate the intermediate with dimethylformamide and obtain the desired product **13** which could be used

without further purification in the next step. A Wittig reaction could then be carried out in excellent yield under mild conditions to form 2-(t-butyldimethylsilyl)-3-hydroxymethyl-4-(2-methoxycarbonylvinyl) furan **14** in 92% isolated yield.

At this stage we investigated a route for converting the primary hydroxyl group into a carboxymethyl function. It became clear that any attempt to carry out a direct reaction on the primary alcohol or its derivative while having the bulky silyl group at position 2 was going to fail. Thus the silyl group was removed using tetrabutylammonium fluoride in tetrahydofuran to give the desired disubstituted furan **15** in 96% yield. It was found that after some trials that the desired transformation could be carried out smoothly in three steps via the mesylate, nitrile and basic hydrolysis. The regular conditions for substituting mesylate by nitrile did not work in our case, but eventually it was found that heating the mesylate dissolved in acetone in presence of potassium cyanide and sodium iodide for 4 hours gave the desired product **16** in 83% yield. Hydrolysis in basic ethanol and esterification with diazomethane yielded the diester **17** in 81% yield.



12 13 14 15 16 17

Scheme V

It is known in furan chemistry[19,20] that electron donating groups at C-3 direct electrophilic attack preferentially to C-2 and electron withdrawing groups at C-3 direct only to C-5. Assuming that the acrylate acts as electron withdrawing and the acetate as electron-donating it was anticipated that formylation by the

Vilsmeier-Haack procedure would occur at C-2. When **17** was treated with phosphorus oxychloride/ dimethylformamide at 80 °C formylation took place and a product could be isolated in 68% yield. Its structure was determined unequivocally by NMR, using nOe technique, to be the 2-formyl-3-propionate-4-acetate **18** (Scheme VI), **isomeric** with the desired compound. At this point we could offer no explanation for the exclusive formation of this unexpected product.

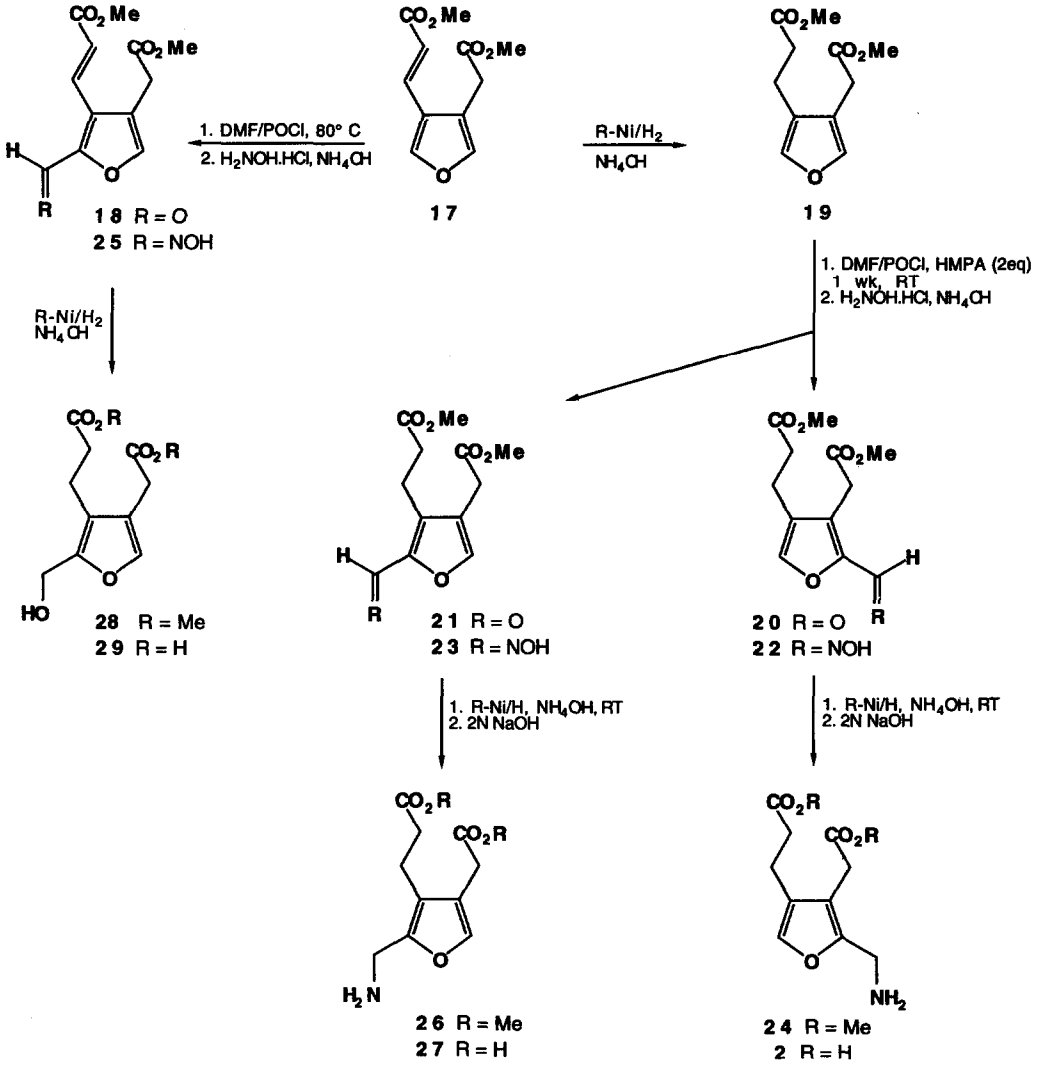
One possibility of influencing the reactivity of the ring in **17** towards electrophilic attack is to reduce the double bond and in this way disrupt the conjugation between the furan ring and the carboxylic ester function. The reduction conditions had to be chosen carefully in order to avoid over-reduction to a tetrahydrofuran. Catalytic reduction of **17** over Raney nickel in the presence of ammonium hydroxide at 35 psi hydrogen, was found to be selective in giving **19** in quantitative yield. Applying Vilsmeir-Haack formylation at 80 °C also led to an unfavorable ratio of the two isomers **20/21** = 2/98 according to NMR analysis. Anderson[21] has shown that the ratio of formylation of pyrrol-3-acetic acid can be altered from 40/60 (C-2/C-5) to 60/40, by addition of hexamethylphosphamide (HMPA) and lowering the temperature. Our results confirm his observations: addition of two equivalents of HMPA at 80 °C led to a ratio of 25/75, and running the reaction for one week at 25 °C led to 33/67 of **20/21**. It is interesting to note that when the reaction was carried out in presence of five equivalents of HMPA and phosphorus oxychloride only the undesired isomer **21** was formed. It is known that electronic and steric factors play important roles in directing electrophilic attack on substituted furans. It can be argued that in our case the three carbon methoxycarbonyl chain, whether unsaturated as in **17** or saturated as in **19** probably stabilizes the intermediate which is formed by attack on carbon 2 more efficiently than the two-carbon methoxycarbonyl chain can stabilize the intermediate resulting from attack on carbon 5.

Separation of isomers **20** and **21** at this stage by chromatography failed. However, the corresponding oxime mixture (**22** and **23**) could be resolved by preparative TLC. Catalytic reduction of **22** over Raney nickel at atmospheric pressure led in nearly quantitative yield to the amino diester furan **24**. Hydrolysis with sodium hydroxide followed by neutralization with Amberlite IRC 50 gave the expected oxa analog of PBG **2** which was tested as a substrate for PBG deaminase.

Since **18** could thus be prepared in high yield two additional compounds were prepared in analogous fashion and tested as deaminase substrates. Isomer **27** was prepared in three steps from **18** by (1) preparation of the corresponding oxime **25**, (2) catalytic reduction at atmospheric pressure over Raney nickel to **26**, (3) hydrolysis with sodium hydroxide to give iso-oxa PBG **27**. Since it is known that the hydroxyl analog of

PBG is a substrate for deaminase[22] its hydroxy-oxa analog **29** was prepared by reduction of **18** to **28** followed by basic hydrolysis.

The three compounds, **2, 27** and **29** were found to be unstable at room temperature and even at 0 °C decomposition had taken place after five weeks. Incubation of each of the oxa PBG analogs **2, 27** and **29** with PBG deaminase **did not lead** to the formation of an enzyme-substrate complex, or any recognizable polymerization product, demonstrating that these oxa-analogs are not substrates of deaminase.



Scheme VI

EXPERIMENTAL

Reagents and Solvents: All chemicals and solvents were reagent grade. Tetrahydrofuran and dichloromethane were distilled over sodium and calcium hydride respectively. Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) were obtained in $CDCl_3$ or D_2O as solvent (unless otherwise stated) on a Varian XL-200 or a Bruker AM-500 equipped with a Bruker Aspect 3000 computer. The chemical shifts are reported in parts per million (ppm) on the δ scale with tetramethylsilane (δ=0), chloroform (δ=7.24) or water (δ=4.62) as internal standards. High resolution mass spectra (HRMS) were recorded on a VG Analytical 70 S spectrometer. Melting points were performed on a Mel-Temp apparatus using capillary tubes and the values reported are uncorrected. Flash chromatography was performed using silica gel 60, 230-400 mesh supplied by Fluka. Preparative and analytical thin-layer chromatography was carried out on Analtech GF silica gel plates (2.0 and 0.25 mm respectively) and Whatman silica gel polyester plates. Medium pressure hydrogenations were performed in a Parr shaker-type 3910 hydrogenation apparatus.

3-[(t-Butyldimethylsilyl)oxymethyl] furan.[5] To a solution of 3-furanmethanol (Aldrich) (20 g; 203 mmol) in DMF (200 mL) were added t-butyldimethylsilyl chloride (32.7 g; 217 mmol) and imidazole (47.8 g; 697 mmol) at room temperature (rt) and the solution stirred for 25 h at rt, ethyl acetate (300 mL) was added and the solution washed with aqueous sodium chloride (4 x 100 mL). The aqueous phase was washed with ethyl acetate (4 x 50 mL), the combined organic phases dried (Na_2SO_4), the solvent was removed under reduced pressure, and the residual product purified by flash chromatography over silica gel, eluting with hexane to give a yield of 42 g (97%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ0.08 (6H, s, $(CH_3)_2Si$); 0.92 (9H, s, $(CH_3)_3CSi$); 4.60 (2H, s, CH_2OSi); 6.36 (1H, d, b-H); 7.34-7.39 (2H, m, b-H). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ -5.20 (2C, $(CH_3)_2Si$); 18.42 ($(CH_3)_3CSi$); 25.94 (3C, $(CH_3)_3CSi$); 57.47 (CH_2OSi); 109.58, 125.79, 139.30, 143.09 (Fur-C).

2-(t-Butyldimethylsilyl)-3-(hydroxymethyl) furan 12. A solution of 3-[(t-butyldimethylsilyl)oxymethyl] furan (49 g; 231.1 mmol) and HMPA (45.5 g; 25.4 mmol) in THF (200 mL) at -78°C under argon was treated dropwise with n-butyllithium (254.2 mmol; 159.0

mL of 1.6 N) in hexane. The solution was stirred at -20°C for 1h and quenched with saturated ammonium chloride solution. The organic phase was separated, the aqueous phase extracted with dichloromethane (4 x 200 mL), dried (Na_2SO_4) and solvents were removed under reduced pressure. The residual product was purified by flash chromatography using silica gel, eluting with 10% ethyl acetate in hexane followed by ethyl acetate-hexane (1:1). The oily product solidfied overnight at 4 °C giving 45 g of **12** in 92% yield. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.27 (6H, s, $(CH_3)_2Si$); 0.88 (9H, s, $(CH_3)_3CSi$); 2.01 (1H, b, OH); 4.55 (2H, s, CH_2OH); 6.45 (1H, d, b-H); 7.55 (1H, d, a-H). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ -5.659 (2C, $(CH_3)_2Si$); 17.37 ($(CH_3)_3CSi$); 26.39 (CH_3CSi); 57.15 (CH_2OH); 110.59, 135.95, 146.79, 154.98 (Fur-C).

2-(t-Butyldimethylsilyl)-4-formyl-3-hydroxymethyl furan 13. To a stirred solution of 2-(t-butyldimethylsilyl)-3-hydroxymethyl furan **12** (46.5 g; 219.3 mmol) in dimethylglycol (250 mL) at -78 °C was added dropwise n-butyllithium (482.5 mmol; 302 mL of 1.6 N) in hexane. The reaction mixture was allowed to warm up to 0 °C and stirred at the same temperature for 45 min. DMF (32 g; 439 mmole) was added dropwise at 0 °C and the reaction mixture stirred further for 1 h. The reaction mixture was quenched with saturated ammonium chloride solution, the organic phase extracted with dichloromethane (4 x 200 mL), dried (Na_2SO_4) and the solvents were removed under reduced pressure. Sample of the product was flash chromatographed on silica gel using as eluant ethyl acetate-hexane (1:4) to give the product, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.28 (6H, s, $(CH_3)_2Si$); 0.87 (9H, s, $(CH_3)_3CSi$); 3.48 (1H, br s, OH); 4.58 (2H, s, CH_2OH); 8.26 (1H, s, a-H); 9.91 (1H, s, CHO). ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ -5.84 (2C, $(CH_3)_2Si$); 17.18 ($(CH_3)_3CSi$); 26.15 (3C, $(CH_3)_3CSi$); 55.48 (CH_2OH); 128.35, 133.9, 158.39 (Fur-C); 186.70 (CHO), identical with the literature values.

2-(t-Butyldimethylsilyl)-3-hydroxymethyl-4-(2-methoxycarbonylvinyl) furan 14. The crude 2-(t-butyldimethylsilyl)-4-formyl-3-hydroxymethyl furan **13** from the previous reaction in anhydrous dichloromethane (200 mL) under argon was treated with methyl (triphenylphosphoranylidiene)acetate (Aldrich) (79.1 g; 237 mmol) and the reaction mixture stirred at rt for 12 h. The solvent was evaporated, and the residual product flash chromatographed on silica gel eluting with ethyl acetate-hexane (4 : 1) to give 59 g of **14** in 92 % yield. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.29 (6H, s, $(CH_3)_2Si$); 0.88 (9H, s, $(CH_3)_3CSi$); 1.90 (1H, br s, OH); 3.74 (3H, s, CO_2CH_3); 4.62 (2H, s, CH_2OH); 6.38 (1H, d, J = 16.1, $CH{=}CHCO_2CH_3$); 7.62 (1H, d, J = 16.1,

$CH{=}CHCO_2CH_3$); 7.84 (1H, s, a-H). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ -5.63 (6H, $(CH_3)_2Si$); 17.16 ($(CH_3)_3CSi$); 26.28 ((3C, $(CH_3)_3CSi$); 51.59 (CO_2CH_3); 55.05 (CH_2OH); 117.97, 134.61 ($CH{=}CHCO_2CH_3$); 122.08, 133.30, 148.69, 159.00 (Fur-C); 167.87 (CO_2CH_3). MS m/e (relative intensities) 297 (m+1)(2), 183 (10), 147 (10), 75 (100), 73 (23).

3-Hydroxymethyl-4-(2-methoxycarbonylvinyl) furan 15. To a stirred solution of 3-hydroxymethyl-4-(2-methoxycarbonylvinyl) furan **14** (20 g; 67.6 mmol) in anhyd THF (50 mL) was added tetrabutylammonium fluoride (81 mmol; 81 mL of 1.0 N) in THF (10 mL) and stirred overnight. Saturated ammonium chloride solution was added, the organic phase evaporated, the product extracted with dichloromethane (4 x 100 mL), dried (Na_2SO_4) and the solvents were removed by reduced pressure. The crude product was flash chromatographed on silica gel eluting with ethyl acetate-hexane mixtures (4 : 1-> 1 : 1) to give 11.8 g of **15** in 96% yield. mp 93-95°C. ^1H NMR ($CDCl_3$, 500 MHz) δ 1.63 (1H, br s, OH); 3.79 (3H, s, CO_2CH_3); 4.68 (2H, s, CH_2OH); 6.36 (1H, d, J = 16.1, $CH{=}CHCO_2CH_3$); 7.46 (1H, d, a-H); 7.61 (1H, d, J = 16.1, $CH{=}CHCO_2CH_3$); 7.68 (1H, d, a-H). ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 1.64 (CO_2CH_3); 55.47 (CH_2OH); 118.24, 134.44 ($CH{=}CHCO_2CH_3$); 121.41, 123.57, 142.56, 145.69 (Fur-C); 167.74 (CO_2CH_3). MS m/e (relative intensities) 182 (23) 151 (19) 150 (18) 123 (20) 122 (100) 121 (21) 94 (25) 66 (14) 65 (17) 51 (14) 39 (22). HRMS Calcd for $C_9H_{10}O_4$ 182.0525, Found 182.0479.

3-Cyanomethyl-4-(2-methoxycarbonylvinyl) furan 16. To a stirred mixture of 3-hydroxymethyl-4-(2-methoxycarbonylvinyl) furan **15** (3.64 g; 20 mmol), anhyd dichloromethane (50 mL) and triethylamine (4.04 g; 40 mmol) at 0°C under argon was added dropwise methanesulfonyl chloride (4.6 g; 40 mmol). After stirring for 15 min at the same temperature, the reaction was stirred at rt overnight. The reaction mixture was washed with cold brine (2 x 20 mL), the organic phase dried (Na_2SO_4), and the solvent was removed by reduced pressure. To the crude product dissolved in anhydrous acetone (50 mL) was added sodium iodide (5.2 g), potassium cyanide (5.2 g; 80 mmol) and the solution was heated at 50°C under argon for 4 h. Brine was added, the acetone removed, and the product extracted with dichloromethane (3 x 50 ML), dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residual product was flash chromatographed on silica gel eluting with ethyl acetate-hexane (1 ; 4) to give 3.2 g of **16** in 83% yield. mp 86-87°C.

^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.64(2H, d, CH_2CN); 3.80 (3H, s, CO_2CH_3); 6.18 (1H, d, J = 16.4, $CH=CHCO_2CH_3$); 7.51 (1H, d, J = 16.4, $CH=CHCO_2CH_3$); 7.55 (1H, d, a-H); 7.73 (1H, d, a-H). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ 13.90 (CH_2CN); 51.87 (CO_2CH_3); 113.74 (CH_2CN); 118.62 ($CH=CHCO_2CH_3$); 133.12 ($CH=CHCO_2CH_3$); 116.38, 120.71, 142.74; 145.38 (Fur- C); 166.90 (CO_2CH_3). MS m/e (relative intensities) 191 (23), 160 (55), 159 (100), 132 (16), 131 (12), 105 (56), 77 (17), 51 (22). HRMS Calcd for $C_{10}H_9NO_3$ 191.0582, Found 191.0590.

3-Methoxycarbonylmethyl-4-(2-methoxycarbonylvinly) furan 17.

3-Cyanomethyl-4-(2-methoxycarbonylvinyl) furan **16** (3.15 g; 16.5 mmol) dissolved in ethanol (40 mL) and 10% aqueous sodium hydroxide (40 mL) was heated to reflux under argon for 10 h. The reaction mixture was acidified (pH 2) with concentrated hydrochloric acid at 0°C, the diacid extracted with ethyl acetate (4 x 30 mL), dried (Na_2SO_4), and the solvents were removed under reduced pressure. To the crude product dissolved in ether (20 mL) was added etheral diazomethane at 0°C and stirred for 30 min and further 30 min at rt. The excess of diazomethane was evaporated and the product flash chromatographed on silica gel eluting with ethyl acetate-hexane (1 : 2) to give 3.0 g of **17** in 81% yield. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.50 (2H, s, $CH_2CO_2CH_3$); 3.64 (3H, s, CO_2CH_3); 3.70 (3H, s, CO_2CH_3); 6.10 (1H, d, J = 16.2, $CH=CHCO_2CH_3$); 7.38 (1H, s, a-H) 7.44 (1H, d, J = 16.2, $CH=CHCO_2CH_3$); 7.63 (1H, s, a-H). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ 29.46 ($CH_2CO_2CH_3$); 51.50 (CO_2CH_3); 52.08 ($CH_2CO_2CH_3$); 117.49 ($CH=CHCO_2CH_3$); 134.27 ($CH=CHCO_2CH_3$); 116.33, 121.55, 142.69, 144.26 (Fur-C); 167.25, 170.79 (2 x CO_2CH_3). MS m/e (relative intensities) 224 (42), 208 (42), 192 (99), 165 (87), 77 (56), 59 (100), 28 (61). HRMS Calcd for $C_{11}H_{12}O_5$ 224.0685, Found 224.0680.

3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl furan 19.

3-Methoxycarbonylmethyl-4-(2-methylcarbonylvinyl) furan **17** (2.1 g; 9.4 mmol) dissolved in methanol (40 mL) with ammonium hydroxide (1 mL) and Raney nickel (1 teaspoon) was hydrogenated at 35 psi for 1 h. The catalyst was filtered, washed with dichloromethane and the filtrate washed with water, dried (Na_2SO_4), and the solvent were removed by reduced pressure. The product was purified by flash chromatography on silica gel eluting with ethyl acetate-hexane (1 : 2) to give 2.12 g of **19** in quatitative yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.58 (2H, t, J = 7.2,

$CH_2CH_2CO_2CH_3$); 2.70 (2H, t, J = 7.2, $CH_2CH_2CO_2CH_3$); 3.42 (2H, s, $CH_2CO_2CH_3$); 3.67 (3H, s, CO_2CH_3); 3.71 (3H, s, CO_2CH_3); 7.21 (1H, d, a- H); 7.35 (1H, d, a-H). ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 18.59 ($CH_2CH_2CO_2CH_3$); 28.92 ($CH_2CO_2CH_3$); 38.49 ($CH_2CH_2CO_2CH_3$); 51.41 (CO_2CH_3); 51.82 (CO_2CH_3); 117.09, 123.37, 139.46, 140.97 (Fur-C); 171.27, 172.98 (2C, 2 x CO_2CH_3). MS m/e (relative intensities) 226 (13), 168 (21), 167 (81), 165 (46), 153 (31), 135 (76), 107 (100), 95 (59). HRMS Calcd for $C_{11}H_{14}O_5$ 226.0841, Found 226.0838.

2-Formyl-4-methoxycarbonylmethyl-3-(2- methoxycarbonylvinyl) furan 18. To a stirred mixture of 3-methoxycarbonylmethyl-4-(2- methoxcarbonyl-vinyl) furan **17** (1.0 g; 4.5 mmol) and DMF (10 mL) at 0 °C under argon was added phosphorous oxychloride (765 mg; 5 mmol) dropwise. After stirring for 30 min at 0 °C, the reaction mixture was heated at 80 °C for 1 h. The reaction mixture was allowed to cool, poured into ice and sodium bicarbonate mixture and stirred for 15 min. The product was extracted with dichloromethane (3 x 20 mL) dried (Na_2SO_4), the solvents were removed by reduced pressure, and the crude product was flash chromatographed on silica gel eluting with ethyl acetate-hexane (2 : 1) to give 710 mg of **18** in 63% yield. mp 70-71°C. ^1H NMR ($CDCl_3$, 200 MHz) δ 3.56 (2H, s, $CH_2CO_2CH_3$); 3.68 (3H, s, CO_2CH_3); 3.74 (3H, s, CO_2CH_3); 6.51 (1H, d, J = 16.3, $CH=CHCO_2CH_3$); 7.67 (1H, s, a-H); 7.81 (1H, d, J = 16.3, $CH=CHCO_2CH_3$); 9.79 (1H, s, CHO). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ 29.49 ($CH_2CO_2CH_3$); 51.95 (CO_2CH_3); 52.51 ($CH_2CO_2CH_3$); 124.79 ($CH=CHCO_2CH_3$); 131.78 ($CH=CHCO_2CH_3$); 119.66, 128.83, 146.45, 149.87 (Fur-C); 166.41, 170.06 (2 x CO_2CH_3); 178.47 (CHO). MS m/e (relative intensities) 252 (1), 221 (5), 194 (11), 193 (100), 105 (5), 77 (7), 59 (8). HRMS Calcd for $C_{12}H_{12}O_6$ 252.0634 Found 252.0644.

2-Hydroxymethyl-3-(2-methoxycarbonylethyl)-4- methoxycarbonylmethyl furan 28. 2-Formyl-4-methoxycarbonylmethyl-3-(2- methoxycarbonylvinyl) furan **18** (100 mg; 0.4 mmol) dissolved in methanol (20 mL) with ammonium hydroxide (1 mL) and Raney nickel (0.5 teaspoon) was hydrogenated and purified as described for **19** to give 72 mg of **28** in 71% yield. ^1H NMR ($CDCl_3$, 200 MHz) δ 2.57 (2H, t, J = 7.2, $CH_2CH_2CO_2CH_3$); 2.75 (2H, t, J = 7.2, $CH_2CH_2CO_2CH_3$); 3.41 (2H, s, $CH_2CO_2CH_3$); .64 (3H, s, CO_2CH_3); 3.71 (3H, s, CO_2CH_3); 4.56 (CH_2OH); 7.32 (1H, s, a-H). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ 18.17 ($CH_2CH_2CO_2CH_3$); 29.29 ($CH_2CO_2CH_3$); 33.90

($CH_2CH_2CO_2CH_3$); 51.73 (CO_2CH_3); 52.04 (CO_2CH_3); 55.37 (CH_2OH); 117.52, 119.81, 140.20, 151.29 (Fur-C); 171.43, 173.75 (2C, 2 x CO_2CH_3). MS m/e (relative intensities) 256 (14), 228 (5), 224 (13), 197 (22), 196 (41), 182 (24), 169 (70), 154 (62), 141 (100), 109 (61), 95 (70), 105 (5), 79 (59), 28 (78). HRMS Calcd for $C_{12}H_{16}O_6$ 256.0947, Found 256.0944.

2-Hydroxymethyl-4-carboxymethyl-3-(2-carboxyethyl) furan 29. 2-Hydroxymethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonyl-methyl furan **28** (25 mg, 0.11 mmol) in 2M piperidine (0.5 mL) was stirred at rt overnight and the solvent was removed at high vacuum and freeze dried. ^1H NMR ($CDCl_3$, 200 MHz) δ 2.43 (2H, t, J = 7.2, $CH_2CH_2CO_2H$); 2.65 (2H, t, J = 7.2, $CH_2CH_2CO_2H$); 3.27 (2H, s, CH_2CO_2H); 4.48 (CH_2OH); 7.19 (1H, s, a-H); 7.45 (1H, b, OH). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ 20.25 ($CH_2CH_2CO_2CH_3$); 32.73 ($CH_2CO_2CH_3$); 37.58 ($CH_2CH_2CO_2CH_3$); 55.28 (CH_2OH); 121.61, 122.52, 138.65, 152.12 (Fur-C); 178.10, 180.32 (2C, 2 x CO_2H).

2-Oxime-4-methoxycarbonylmethyl-3-(2- methyoxycarbonylvinyl) furan 25. To a stirred solution of 2-formyl-4-methoxycarbonylmethyl-3-(2-methoxycarbonylvinyl) furan **18** (100 mg, 0.40 mmol) in methanol (3 mL) was added hydroxylamine hydrochloric (41.7 mg, 0.6 mmol), sodium acetate (49.2 mg, 0.6 mmol), and the reaction was stirred under argon at rt overnight. The reaction mixture was diluted with water (10 mL), extracted with dichloromethane (3 x 20 mL), dried (Na_2SO_4), and the solvents were removed under reduced pressure to give **25** in quantitative yield. mp 126-128°C. ^1H NMR ($CDCl_3$, 500 MHz) δ 3.60 (2H, s, $CH_2CO_2CH_3$); 3.74 (3H, s, CO_2CH_3); 3.80 (3H, s, CO_2CH_3); 6.23 (1H, d, J = 16.5, $CH=CHCO_2CH_3$); 7.51 (1H, s, a-H) 7.74 (1H, d, J = 16.5, $CH=CHCO_2CH_3$); 8.21 (1H, s, CH=NOH), 8.64 (1H, b s, OH). ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 29.93 ($CH_2CO_2CH_3$); 51.88 (CO_2CH_3); 52.36 (CO_2CH_3); 20.30.55 ($CH=CHCO_2CH_3$); 133.55 ($CH=CHCO_2CH_3$); (119.30, 125.83, 140.04, 144.74 (Fur-C); 139.08 (CH=NOH); 167.20, 170.62 (2C, 2 x CO_2CH_3). MS m/e (relative intensities) 267 (0.1), 209 (18), 208 (100), 132 (29), 59 (21). HRMS Calcd for $C_{12}H_{13}NO_6$ 267.0729, Found 267.0740.

2-Formyl-4-(2-methyoxycarbonylethyl)-3-methoxycarbonylmethyl furan 20. To a stirred solution of 4-(2-methoxycarbonylethyl)-3-methoxycarbonyl-methyl furan **19** (117 mg; 0.5 mmol) in

DMF (2 mL) at 0°C under argon was added phosphorous oxychloride (84.3 mg; 0.55 mmol) dropwise followed by addition of HMPA (187 mg, 1.0 mmol). The reaction was stirred at rt for 7 days, worked-up and purified as described for **18** to give 100 mg of isomeric mixture of **20** and **21** in a 1:2 ratio respectively. **20**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.60 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 2.72 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 3.69 (3H, s, CO_2CH_3); 3.73 (3H, s, CO_2CH_3); 3.89 (2H, s, $CH_2CO_2CH_3$); 7.52 (1H, s, a-H); 9.76 (1H, s, CHO). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ 18.13 ($CH_2CH_2CO_2CH_3$); 20.94 ($CH_2CO_2CH_3$); 28.77 ($CH_2CH_2CO_2CH_3$); 51.64 (CO_2CH_3); 52.32 (CO_2CH_3); 127.24, 134.67, 144.47, 149.84 (Fur-C); 170.66, 172.66 (2C, 2 x CO_2CH_3).179.29 (CHO). **21**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.67 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 3.03 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 3.58 (2H, s, $CH_2CO_2CH_3$); 3.66 (3H, s, CO_2CH_3); 3.74 (3H, s, CO_2CH_3); 7.65 (1H, s, a-H); 9.78 (1H, s, CHO). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ 18.13 ($CH_2CH_2CO_2CH_3$); 28.54 ($CH_2CO_2CH_3$); 33.36 ($CH_2CH_2CO_2CH_3$); 51.73 (CO_2CH_3); 52.23 (CO_2CH_3); 120.90, 134.47, 145.78, 149.14 (Fur-C); 170.75, 172.75 (2C, 2 x CO_2CH_3).179.03 (CHO). MS m/e (relative intensities) 254 (26), 222 (73), 223 (49), 195 (59), 194 (87), 163 (36), 138 (35), 135 (100), 79 (60), 77 (55), 59 (48), 28 (75). HRMS Calcd for $C_{12}H_{14}O_6$ 254.0790, Found 254.0796.

2-Oxime-4-(2-methyoxycarbonylethyl)-3-methoxycarbonylmethyl furan 22. To a mixture of the isomeric 2-formyl furan **20** and **21** (60 mg, 0.24 mmol) in methanol (5 mL) was added hydroxylamine hydrochloric (24.6 mg, 0.35 mmol), sodium acetate (29.1 mg, 0.35 mmol) and the reaction was stirred under argon at rt overnight. The reaction mixture was diluted with water (10 mL) and the product extracted with dichloromethane (3 x 20 mL), dried (Na_2SO_4), and solvents were removed under reduced pressure to give in quantitative yield a mixture of **22** and **23**. Repeated preparative thin layer chromatography eluting with hexane-ethyl acetate (3 : 1) gave 15 mg of **22**. mp 110-112°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.56 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 2.70 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 3.57 (2H, s, $CH_2CO_2CH_3$); 3.66 (3H, s, CO_2CH_3); 3.67 (3H, s, CO_2CH_3); 7.24 (1H, s, a-H); 8.02 (1H, s, CH=NOH), 8.64 (1H, b s, OH). ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ 18.64 ($CH_2CH_2CO_2CH_3$); 29.28 ($CH_2CO_2CH_3$); 33.57 ($CH_2CH_2CO_2CH_3$); 51.81 (CO_2CH_3); 52.37 (CO_2CH_3); 19.30, 125.83, 140.04, 144.74 (Fur-C); 140.59 (CH=NOH); 170.73, 173.13 (2C, 2 x CO_2CH_3). MS m/e (relative intensities) 269 (29), 238 (28),237 (47) 210 (18), 178 (15), 150 (23) 136 (18), 77 (22), 64 (35), 59 (100), 57 (36). HRMS Calcd for $C_{12}H_{15}NO_6$ 269.0899, Found 269.0890.

2-Aminomethyl-3-(2-methoxycarbonylethyl)-4- methoxycarbonylmethyl furan 24.

2-Oxime-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethyl furan **22** (20 mg; 0.7 mmol) dissolved in methanol (3 mL) with ammonium hydroxide (20 drops) and Raney nickel (0.5 teaspoon) was hydrogenated at atm pressure for 1 h and purified as described for **19**. The product **24** was obtained in nearly quantitative yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.56 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 2.68 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 3.36 (2H, d d, $CH_2CO_2CH_3$); 3.69 (6H, s, CO_2CH_3); 4.44-4.47 (2H, m, CH_2NH_2); 6.86 (1H, s, CH_2NH_2); 7.18 (1H, s, a-H). ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 18.86 ($CH_2CH_2CO_2CH_3$); 28.23 ($CH_2CO_2CH_3$); 35.54 ($CH_2CH_2CO_2CH_3$); 40.98 (CH_2NH_2); 51.72 (CO_2CH_3); 113.40, 122.43, 139.43, 142.78 (Fur-C); 170.02, 172.91 (2C, 2 x CO_2CH_3). MS m/e (relative intensities) 255 (1), 223 (100), 222 (77), 192 (19), 164 (20), 150 (47), 121 (22), 77 (20), 28 (59). HRMS: Calcd for $C_{12}H_{17}NO_5$ 225.1107, Found 225.1117.

2-Aminomethyl-3-(2-carboxyethyl)-4-carboxymethyl furan 2.

2-Aminomethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl furan **24** (17.0 mg, 0.12 mmol) in methanol (1 mL) was treated with 2N sodium hydroxide (0.5 mL) and stirred at rt overnight. Amberlite IRC 50 was added and the reaction mixture was filtered at neutral pH, the filtrate was freeze dried to give **2** in nearly quantitative yield. ^{1}H NMR: (D_2O, 500 MHz) δ 2.19 (2H, t, J = 7.3, $CH_2CH_2CO_2H$); 2.34 (2H, t, J = 7.3, $CH_2CH_2CO_2H$); 3.12 (2H, s, CH_2CO_2H); 3.92 (2H, s, CH_2NH_2); 7.08 (1H, s, a-H); 8.20 (2H, b s, CH_2NH_2). ^{13}C NMR: (D_2O, 125.7 MHz) δ 18.62 ($CH_2CH_2CO_2H$); 30.52 (CH_2CO_2H); 33.07 (CH_2NH_2); 35.64 ($CH_2CH_2CO_2H$); 119.56, 124.54, 138.61, 141.90 (Fur-C); 178.07, 181.07 (2 x,CO_2H).

2-Aminomethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl furan 26.

2-Oxime-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl furan **25** (34.4 mg; 0.13 mmol) was reduced in methanol (2 mL), Raney nickel (0.25 teaspoon) and ammonium hydroxide (10 drops) as described for **24**. The product **25** was obtained in nearly quantitative yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.50 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 2.70 (2H, t, J = 7.3, $CH_2CH_2CO_2CH_3$); 3.41 (2H, s, $CH_2CO_2CH_3$); 3.65 (3H, s, CO_2CH_3); 3.71 (3H, s, CO_2CH_3); 3.77 (2H, s, CH_2NH_2); 7.28 (1H, s, a-H); ^{13}C NMR ($CDCl_3$, 50.8 MHz) δ ($CDCl_3$, 125.7 MHz) δ 18.43 ($CH_2CH_2CO_2CH_3$); 29.41

($CH_2CO_2CH_3$); 34.53 ($CH_2CH_2CO_2CH_3$); 37.27 (CH_2NH_2); 51.61 (CO_2CH_3); 52.07 (CO_2CH_3); (117.49, 117.63, 1139.40, 152.83 (Fur-C); 171.58, 173.13 (2C, 2 x CO_2CH_3). MS m/e (relative intensities) 255 (34), 207 (14), 181 (14), 168 (100),44 (19), 32 (26), 28 (12), 77 (20), 28 (59). HRMS Calcd for $C_{12}H_{17}NO_5$ 255.1107, Found 255.1107

2-Aminomethyl-3-(2-carboxyethyl)-4-carboxymethyl furan 27.

2-Aminomethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl furan **26** (31.0 mg, 0.12 mmol) in methanol (1 mL) was treated with 2N sodium hydroxide (0.5 mL) as described for **2** to give **27** in nearly quantitative yield. ^1H NMR (D_2O, 500 MHz) δ 2.17 (2H, t, J = 7.3, $CH_2CH_2CO_2H$); 2.44 (2H, t, J = 7.3, $CH_2CH_2CO_2H$); 3.10 (2H, s, CH_2CO_2H); 3.95 (2H, s, CH_2NH_2); 7.15 (1H, s, a-H); 8.20 (2H, b s, CH_2NH_2). ^{13}C NMR (D_2O, 125.7 MHz) δ 19.62 ($CH_2CH_2CO_2H$); 32.02 (CH_2CO_2H); 34.22 (CH_2NH_2); 37.23 ($CH_2CH_2CO_2H$); 121.04, 124.77, 141.54, 142.45 (Fur-C); 179.86, 181.95 (2 x, CO_2H). Calcd for $C_{10}H_{15}O_5$ 227, Found m/e 228 (m+1).

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REFERENCES

1. Willen, S. H.; Shen, D.; Licata, J. M.; Baldwin, E.; Russell, C.S. *Heterocycles* **1984**, *22*, 1747.
2. Vogel, E.; Hass, W.; Knip, J.; Lex, J.; Schmikler, H. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*,406.
3. Schultz, A. G.; Motyka, L.A. *J. Am. Chem. Soc.* **1982**, *104*, 5800.
4. Corey, E. J.; Crouse, D. N.; Anderson, J. E. *J. Org. Chem.* **1975**, *40*, 2140.
5. (a) Bures, E. J.; Keay, B. A. *Tetrahedron Lett.* **1987**, *28*, 5965. (b) *Tetrahedron Lett.* **1988**, *29*, 1247. (c) Spinazze, P. G.; Keay, B. A. *Tetrahedron Lett.* **1989**, *30*, 1765.
6. (a) Weis, C. D. *J. Org. Chem.* **1962**, *27*, 3520. (b) Ansell, M. F.; Caton, M. P. L.; North, P. C. *Tetrahedron Lett.* **1981**, *22*, 1727. (c) Konig, H.; Graf, F.; Webernodorfer, V. *Liebigs Ann. Chem.* **1981**, 668.
7. Barton, D. H. R.; Dawes, C. C.; Franceschi, F.; Fogloi, M.; Ley, S. V.; Magnus, P. D.; Mitchell, W. L., Temperelli, A. *J. Chem. Soc. Perkin Trans. 1* **1980**, 643.
8. Reich, H. J.; Olson, R. E. *J. Org. Chem.* **1982**, *52*, 2315.
9. Danheiser, R. L.; Stoner, E. J.; Koyama, J.; Yamashita, D. S.; Klade, C. A. *J. Am. Chem. Soc.* **1989**, *111*, 4407.
10. Marshall, J. A.; Xiao-Jun, W. *J. Org. Chem.* **1991**, *56*, 960.
11. D'Alelio, G. F. *U. S. Pat.* 3664992 **1972**.
12. Davies, G. M.; Davies, P. S. *Tetrahedron* **1972**, *33*, 3507.
13. Kenner, G. W.; Smith, K. M.; Unsworth, J. F. *J. Chem. Soc. Chem. Commun.* **1973**, 43.
14. Wollmann, T.; Franck, B. *Angew Chem. Int. Ed. Engl.* **1984**, *23*, 226.
15. Myrboh, B.; Ila, H.; Junjappa, H. *Synthesis* **1981**, 126.
16. Jacobsen, H. J.; Lawesson, S. O. *Tetrahedron* **1965**, *21*, 3331.
17. Scheinkonig, J.; Puchta, V.; Spiteller, G. *Liebigs Ann. Chem.* **1989**, 449.
18. Kawashima, M.; Sato, T.; Fujisawa, T. *Tetrahedron* **1989**, *45*, 403.
19. Sargent, M. W.; Dean, F. M. in *Comprehensive Heterocyclic Chemistry*, Katritzky, A. R. Ed., Pergamon Press, New York, 1987, vol. 4, p.599.
20. Marino, G. *Adv. Heterocycl. Chem.* **1971**, *13*, 235.
21. Faber, K.; Anderson, H. J.; Loader, C. E.; Daley, A. S. *Can. J. Chem.* **1984**, *62*, 1046.
22. Battersby, A. R.; Fookes, C. J. R.; Matcham. G. W. J.; McDonald, E.; Gustafson-Potter, K. E. *J. Chem. Soc. Chem. Commun.* **1979**, 316.